with high affinity and exhibit a robust open probability ~0.4. Substituting dual alanines for the 'IQ' residues in the IQ element significantly weakens apocalmodulin binding, and markedly diminishes peak open probability (~0.1). Overexpressing apocalmodulin fully rescues open probability, demonstrating that the effects on opening reflect apocalmodulin binding *per se*. This extensive structural and functional similarity substantiates a striking conservation of calmodulin regulation across Nav and Cav channels, joint investigation of which now presents as a genuinely synergistic endeavor.

## Platform: Optical Spectroscopy: CD, UV-VIS, Vibrational, Fluorescence

**2483-Plat**
**Ratiometric Bioluminescence-Based Zinc Biosensor with Nanomolar Sensitivity**
Evgenia G. Matveeva[1], Graham Franke[1], Leslie Bourne[1], Andrea K. Stoddard[2], Carol A. Fierke[2], **Richard B. Thompson**[1].
[1]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, USA, [2]Department of Chemistry, University of Michigan, Ann Arbor, MI, USA.
In recent years, we and other workers have developed fluorescence-based sensors and biosensors for Zn2+ and other free metal ions in solution, which have proven valuable as tools for the study of the biology of metal ions. Using the sensors in some applications such as in vivo molecular imaging has been challenging, owing to the difficulty of exciting fluorophores deep in tissues which scatter and absorb exciting light effectively. One approach used by investigators to minimize this problem is the use of bioluminescent labels for in vivo imaging, which perforce require no exciting light. We have employed a bioluminescence resonance energy transfer approach with our carbonic anhydrase-based zinc biosensor to produce a ratiometric bioluminescence-based zinc biosensor.

**2484-Plat**
**From Charge State to Isosurfaces to Spectra: Unraveling the Mystery of Lys-Trp Dipeptide Fluorescence**
**Azaria Eisenberg**, Laura Juszczak.
Brooklyn College, Brooklyn, NY, USA.
The quest for factors controlling the partition of excitation energy between fluorescence and nonradiative processes in tryptophan is of perennial interest to spectroscopists because its fluorescence has distinct intensity, color and environmental sensitivity. Solving the relationship between specifics of molecular environment and characteristics of tryptophan fluorescence would provide the key for emission spectra interpretation of a wide variety of proteins. Our quantum mechanical calculations and molecular modeling reveal substantial differences in ground state isosurface charge distribution on the indole ring for Lys-Trp dipeptides when backbone and residue charge is varied. These isosurfaces represent the superposition of all ground state molecular orbital contributions to charge distribution and thereby provide a visual representation of the contributions of all relevant orbitals. Comparison of Lys-Trp species isosurfaces with experimentally derived quantum yields and fluorescence lifetimes reveals a correlation: high $\pi$-electron density on the indole ring is associated with high quantum yield and long average lifetime, both of which are found in the zwitterionic and anionic states. Conversely, low quantum yield and short average lifetime is associated with low, uneven $\pi$-electron density on the indole ring, which is found in the cationic and highly anionic state where the indole amine is deprotonated. This interpretation explains fluorescence results for tryptophan-containing proteins. Where x-ray crystal structures have shown proteinaceous tryptophans to be hydrogen bonded at the indole amine, very low quantum yields are observed, and isosurfaces resembling those with a deprotonated indole amine are anticipated. Thus isosurfaces reveal losses in $\pi$-electron density over the indole ring or a loss of aromaticity for low fluorescing tryptophans. Isosurfaces are the key to solving the relationship between molecular environment and tryptophan fluorescence.

**2485-Plat**
**Real-Time Quantification of Time-Gated Autofluorescence Spectrum Shape to Track Mitochondrial Metabolism**
**Paul Urayama**, Jeff Maltas, Zac Long, Arthur Oliva, Jeff Folz, Lana Amer, Dylan Palo.
Physics, Miami University, Oxford, OH, USA.
Recent studies by various groups report the use of NADH as an intrinsic optical biomarker and metabolic indicator. Using measurements of excited-state lifetime or anisotropy decay, different forms of intracellular NADH were identified and monitored, with the response of NADH to metabolic conditions being more complex than a simple concentration change. Recently, we report an approach for the real-time tracking of UV-excited autofluorescence based on the rapid quantification of spectrum shape. Here, we show that nanosecond-gated spectral acquisition - combined with spectrum-shape quantification (such as with spectral phasor analysis) - can be used to track the physiologic response of a cellular system (*Saccharomyces cereviseae*) to the addition of mitochondrial functional modifiers (e.g., cyanide) and metabolic substrates (e.g., ethanol and glucose). We demonstrate that time-gated detection allows for the rejection of short excited-state-lifetime emission. We observe that the spectrum shape of long excited-state-lifetime autofluorescence exhibits a different response to chemical additions than that of the time-integrated autofluorescence, even allowing for the discrimination between nominally similar responses. Results are consistent with the existence of multiple NADH forms, as changes in autofluorescence spectrum shape cannot be accounted for by a two spectra model. Time-gated spectroscopy combined with rapid spectrum-shape analysis may lead to new and useful approaches for the real-time tracking of cellular metabolic state.

**2486-Plat**
**Nanoscale Infrared Spectroscopy of Biological Systems**
**Eoghan Dillon**, Mike Lo, Kevin Kjoller, Qichi Hu, Roshan Shetty.
Anasys Instruments, Santa Barbara, CA, USA.
Infrared microspectroscopy is a popular technique for investigating biological structures. It is relatively simple to use, and considered to be a non-destructive technique. By combining atomic force microscopy and infrared spectroscopy (AFM-IR), it is possible to resolve chemical differences on the scale of ~100 to 200 nm, which often reveals information that could not have been obtained with conventional infrared microspectroscopy. Currently, AFM-IR spectroscopy has the ability to collect IR spectroscopic information below the diffraction limit with lateral resolution of ~ 100 nm. However, there are still some limitations that prevent its use on many important nanoscale systems. One of the main limitations is the thickness of the sample required for examination ($>$ 100 nm). Overcoming these limitations has a dramatic impact by enabling widespread use of nanoscale IR spectroscopy for spatially resolved chemical characterization. The use of a quantum cascade laser (QCL) as the IR source significantly increases the sensitivity of AFM-IR. The QCL has repetition rates 1000 times higher than previous lasers used for AFM-IR. This allows the ability to pulse the laser at the resonant frequency of the AFM cantilever giving rise to a high IR sensitivity mode referred to as resonance enhanced infrared nanospectroscopy (REINS). Additional enhancement of the AFM-IR signal results when a gold-coated AFM tip is used, producing a ''lightning-rod'' effect which enhances the intensity of the exciting electric field at the tip. Furthermore, if the sample is deposited onto a gold substrate, the local electric field is further enhanced allowing for chemical identification of samples as thin as 25 nm. In this presentation we will demonstrate the effectiveness of the AFM-IR technique on several biological systems, including; IR spectroscopy of a monolayer film of Halobacterium salinarium on a gold substrate and IR chemical imaging of Streptomyces bacteria.

**2487-Plat**
**Flavonols as Luminescent Probes of Water Activity in Foods and Pharmaceuticals**
Siyu (Suzie) Wang, An N. Le, Maria G. Corradini, **Richard D. Ludescher**.
Food Science, Rutgers University, New Brunswick, NJ, USA.
Flavonols are naturally occurring antioxidants with complex photophysical properties. Their emission sensitivity to polarity and hydrogen bonding ability of the local environment suggests their potential as luminescent probes for water activity. Water activity, aw, is an important parameter for ensuring food safety and quality, as it is a determining factor for microbial growth and biochemical reactions.
This study focused on 3-hydroxyflavone (3HF), a synthetic molecule that constitutes the backbone of naturally occuring flavonols. However, the photophysical properties and sensitivity to aw of other flavonoid glycosides were also explored. All flavonols were tested in binary solvent mixtures of different aw. 3HF is an excited-state intramolecular proton-transfer probe that exhibits dual fluorescent emission bands corresponding to its normal ($\lambda$em = 405nm) and photo-induced tautomeric ($\lambda$em = 525nm) form. The normalized intensity of the photo-induced tautomer was sensitive to aw of binary mixtures, with a marked decrease in intensity at aw $>$ 0.8, likely due to progressive aggregation of 3HF molecules. Additional sensitivity to aw was observed in terms of the location of the emission bands. The difference in wavelength between the normal (N) and tautomer (T) forms decreased monotonically at aw $>$ 0.4 due to a hypsochromic shift of the tautomer band. The relative position of the T* towards N* band as a function of aw was modelled using a log-logistic function. A critical aw value above which the sensitivity of 3HF significantly increased was estimated based on the model.
Quercetin and additional flavonoid glycosides were also responsive to changes in aw; e.g., quercetin's fluorescence intensity decreased at aw $>$ 0.6.
Although additional validation of these probes in model food systems is required, the available data support the potential use of flavonols as probes of aw in foods and edible pharmceuticals.